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THE EFFECTS OF FATTY ACID AND SHRIMP MEAL COMPOSITION OF PREPARED DIETS ON GROWTH OF JUVENILE SHRIMP, *Penaeus stylirostris*

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ABSTRACT

Pelletized diets containing different amounts of sun-dried shrimp meal and brewer's yeast were tested. A relationship was observed between the content of shrimp meal and brewer's yeast in the diets and increased growth of juvenile shrimp, *Penaeus stylirostris*. Data suggested that in feeds containing 5% squid meal, up to half the shrimp meal can be replaced by brewer's yeast. These feeds should thus contain at least 15% shrimp meal and less than 15% brewer's yeast. A quadratic correlation was found between the rate of growth of juvenile shrimp and the percentage of linoleic acid in the diets. The best nutritional response should be obtained in rations containing 14.5% linoleic acid. The same type of relation was observed for the ratio of the linolenic series ($\omega3$)/linoleic acid ($\omega6$) with the ratio of 1.18 giving the best nutritional response for the diets tested in this study.

INTRODUCTION

Shrimp meal has been used as animal protein and fatty acid source in feeds for several species of shrimps (Sick et al. 1972; Balazs et al. 1973; Forster 1972; Fenucci and Zein-Eldin 1979; Fenucci et al. 1980). Because shrimp meal is becoming more expensive and difficult to obtain, several pelletized feeds were prepared in which this ingredient was replaced by varying percentages of brewer's yeast. Also, 15% corn meal was substituted for an equivalent amount of rice bran in the diets to determine if corn meal can be used in pelletized feeds both as a cheap source of energy and a binding agent.

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Since it has been suggested that fatty acids, mainly the linolenic and linoleic series, are important in shrimp nutrition (Shewbart and Mies 1973; Guary et al. 1976; Colvin 1976; Kanazawa et al. 1977a,b; Bottino et al. 1980), fatty acid profiles of the diets were determined and the relation between feed composition (in terms of fatty acids) and the growth of the juvenile shrimp *Penaeus stylirostris* was evaluated.

MATERIALS AND METHODS

Juvenile *P. stylirostris* were laboratory hatched and reared. Experimental animals were weighed individually to the nearest 0.01 g, both initially and at the end of the experiment. Fifteen shrimp, each weighing approximately 0.7 g, were placed in each 60-liter aquarium prepared with undergravel filter beds consisting of oyster shell and sand (Zein-Eldin and Meyers 1973). The tanks were filled with natural seawater which was first passed through a 5 μ filter and then through a quartz ultraviolet sterilizer. Aquaria were placed in rooms with temperature controlled at 28±1°C and a light cycle of 12 hours light and 12 hours dark. Water temperature in the aquaria ranged from 27 to 30°C and salinity from 28 to 30 ppt during the experiment.

The shrimp were fed for 14 days with one of the several pelletized feeds listed in Table 1. The pelletized feeds were prepared using the ingredients and techniques described by Fenucci and Zein-Eldin (1979). The initial feeding level was approximately 5% of biomass per day, given in two preweighed feedings. The amount of food given was adjusted daily, so that the animals ate until satiation. Uneaten feed was removed daily, then dried and weighed to determine the exact weight of food ingested. Exuviae and dead shrimp were also removed daily and recorded.

The feed conversion rates were calculated as amount of food eaten divided by the increase in biomass. Rates calculated on the basis of corrected biomass would be smaller than those reported here.

To determine whether the differences in increase of mean weight and change in biomass of shrimp were statistically significant, Student's t-test was used, following a test for homoscedasticity of variances (Cochran method, Gmurman 1974) and an analysis of variance. For the comparison of the increase in biomass between diets K and 30, a modification of the Student's t-test was used (Sokal and Rohlf 1969) because of the heteroscedasticity of the variances.

DESIGN AND ANALYSIS OF FEEDS

Diet K (Fenucci and Zein-Eldin 1979) containing 31.5% of shrimp meal was used as the standard diet. Using this ration as a basis, a series of feeds was formulated replacing shrimp meal with different amounts of yeast (5, 15.7, 26.5 and 31.5% of the total diet, Table 1). The amount of protein in each formulated feed was very similar, since yeast was 40.0% crude protein and the shrimp meal, 41.0%.

The total protein of rations and meals was determined by semi-micro Kjeldahl analysis (Barnes 1959). Carbohydrates were determined by a colorimetric method (Dubois et al. 1956). Total lipids were analyzed using the method described by Hanson and Olley (1963). The relative fatty acid composition of the diets was determined in a gas-liquid chromatograph (Varian 3700) fitted with a flame ionization detector and stainless steel column, packed with 15% of diethylenglycol succinate (DEGS) on Chromosorb W 80-100, at 180°C column temperature. Fatty acids in the chromatogram were identified by comparison of their retention times with known fatty acids. The percentage of each acid was calculated by measuring the area of the peaks (Moreno 1977; Moreno et al. 1979).

Table 1. Percentage Composition of Formulated Feeds

Component	Diet					
	25	26	27	28	K[a]	30
Shrimp meal	26.5	15.7	5.0	--	31.5	31.5
Brewer's yeast	5.0	15.8	26.5	31.5	--	--
α-Soy	3.0	3.0	3.0	3.0	3.0	3.0
Fish meal	8.0	8.0	8.0	8.0	8.0	8.0
Rice bran	44.0	44.0	44.0	44.0	44.0	29.0
Squid meal	5.0	5.0	5.0	5.0	5.0	5.0
Corn meal	--	--	--	--	--	15.0
Fish solubles	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin mix	2.0	2.0	2.0	2.0	2.0	2.0
Alginate	2.5	2.5	2.5	2.5	2.5	2.5
Na hexametaphosphate	1.0	1.0	1.0	1.0	1.0	1.0
Lecithin	1.0	1.0	1.0	1.0	1.0	1.0
Protein %[b]	34.3	35.8	33.9	31.5	31.3	32.9
Lipids %	4.2	5.6	6.4	5.8	4.1	5.7
Carbohydrates %	20.6	25.1	28.7	25.0	15.9	35.0
Moisture %	6.1	5.8	4.1	3.1	5.9	4.4

[a]Source of components as in Zein-Eldin and Meyers (1973) except for vitamin Mix-AIN vitamin Mixture 76[a] of ICN nutritional Biochemicals containing no ascorbic acid, choline or inositol.

[b]Percentages of lipids, proteins and carbohydrates on dry weight bases from triplicate determinations.

RESULTS

After the 14-day feeding trial, greatest increases in mean weight were obtained using diets 25, 26 and K containing 26.5, 15.7 and 31.5% shrimp meal respectively. These increments were significantly larger than those shown by shrimp fed rations 27 and 28, with lower amounts of shrimp meal (Table 2). These differences could be due either to an inadequate amount of shrimp meal or too much brewer's yeast in the diet. Excellent results were obtained with all diets, since the lowest increase was 88.5% for diet 28 and the maximum was 136.8% for ration 26.

As mentioned above, Diet 30 was prepared by replacing 15% of rice bran in diet K by an equal percentage of corn meal. There was no significant difference between these two feeds with regard to the increase in mean weight (Table 2), although growth was slightly lower and the SEM was higher. Survival ranged from 75 to 90.7% and the food conversion rates varied between 3.4 for Diet 26 and 5.9 for Diet 30, with no significant differences.

Table 2. Growth and Survival of *P. stylirostris* Fed Various Rations for 14 Days. Average from triplicate tanks, each containing 15 shrimp. Weight values are means ± SEM.

Diet	Weight (g) Initial	Final	Change	% Change	% Survival	Amount fed (g)	C/R[a]
25	0.73 ± 0.010	1.65 ± 0.069	0.92 ± 0.059	126	75	39.4	5.3
26	0.71 ± 0.005	1.68 ± 0.095	0.97 ± 0.095	137	89	38.9	3.4
27	0.74 ± 0.003	1.42 ± 0.076	0.68 ± 0.078[b]	93	82	33.6	5.4
28	0.73 ± 0.006	1.37 ± 0.085	0.64 ± 0.080[b]	88	91	30.4	4.1
K	0.71 ± 0.012	1.67 ± 0.069	0.96 ± 0.073	135	84	40.4	4.0
30	0.72 ± 0.007	1.56 ± 0.151	0.83 ± 0.166	116	82	34.1	5.9

[a]Food Conversion Rate (amount fed in grams divided by increase in shrimp weight in grams).

[b]Values are significantly less (<0.05) than change of weight for other 4 diets.

No correlations were found between the increase in mean weight of the juvenile *P. stylirostris* (Table 2) and the relative percentage of fatty acids from the ω3 series nor the amount of 20:5 ω3 and 22:6 ω3 in the diets (Table 3). However, there is a quadratic correlation between shrimp growth and the percentage of linoleic acid, a major unsaturated fatty acid of the ω6 series in these diets. For the values tested (12.4 to 18.4%) the change follows a pattern represented by the expression $y = -4.651 + 0.756\,x - 0.026\,x^2$, with a correlation coefficient $r = 0.758$ for 12 degrees of freedom. According to this equation, the best nutritional response should be obtained in rations containing 14.5% linoleic acid (Fig. 1).

Also, a quadratic relation was determined between the gain in mean weight and the ratio % ω3/% linoleic acid (relative percentage of acids from the ω3 family to the relative percentage of linoleic acid); the data fit the model given by $y = 1.683 + 4.51\,x - 1.906\,x^2$ with $r = 0.694$ for 12 degrees of freedom. Under the experimental conditions used in this study, a diet having the ratio of 1.18 for ω3 fatty acids to 1 of linoleic acid should give the best nutritional response (Fig. 2).

DISCUSSION

Both shrimp meal and yeast have been used by several authors as ingredients in prepared feeds for shrimp nutrition. Balazs et al. (1973) prepared an excellent diet for *P. japonicus* with 45% of shrimp meal and 5% of brewer's yeast while for the same species Deshimaru and Kuroki (1974) prepared a control feed having 13.8% of shrimp meal and 18.4% of brewer's yeast. For *P. aztecus*, the best increase in biomass was found by Sick et al. (1972) with a ration containing 69.5% shrimp meal while Fenucci and Zein-Eldin (1979) and Zein-Eldin and Corliss (1979) obtained good growth, in several nutritional trials, with diets which had 31.5% shrimp meal. For post-larval and juvenile stages of *P. stylirostris* and *P. californiensis*, Colvin and Brand (1977) tested several diets comprising 15-30% shrimp meal.

Table 3. Relative Fatty Acid Composition (%) of Diets Fed to *Penaeus stylirostris*

Fatty acid	Diets 25	26	27	28	K
14:0	4.1	4.6	3.6	3.2	5.2
14:1	2.3	0.8	---	0.9	0.6
15:0	1.4	---	0.5	1.0	1.2
?	---	---	---	---	0.5
16:0	19.9	19.1	21.3	22.7	19.1
16:1 ω7	4.7	7.0	5.7	5.5	6.3
17:0	1.3	1.5	0.7	1.2	2.6
17:1	1.0	1.3	0.5	0.7	1.7
18:0	4.9	7.2	4.8	3.9	7.1
18:1 ω9	16.9	20.3	21.8	21.8	17.0
18:2 ω6	12.4	15.9	17.5	18.4	15.2
18:3 ω3	2.9	2.4	2.8	2.3	3.1
20:1	4.0	2.7	2.6	1.9	1.9
18:4 ω3	1.6	2.3	1.8	1.5	1.4
20:2	1.6	0.7	---	---	1.0
20:3	1.4	---	0.2	---	0.3
20:4 ω6	1.7	1.2	1.2	0.7	1.6
22:1	---	---	---	0.5	---
20:4 ω3	0.9	0.7	0.7	---	0.7
20:5 ω3	6.0	4.7	6.7	5.8	5.5
24:0	1.6	1.6	0.5	1.0	1.1
22:4 ω3	1.2	---	0.4	0.8	0.9
22:5 ω3	1.2	0.5	0.2	0.8	0.5
22:6 ω3	4.1	4.2	3.5	4.0	4.2
Total % ω3	17.9	14.8	16.1	15.2	16.3
Ratio: % ω3/% 18:2 ω6	1.33	0.93	0.92	0.82	1.07
Total % 20:5 ω3 + 22:6 ω3	10.1	8.9	10.2	9.8	9.7

The results obtained in this experiment with juvenile *P. stylirostris* fed diets 25, 26 and K, with percentages of shrimp meal ranging from 15.7 to 31.5%, showed no significant differences in increase in mean weight; these results are better than those obtained with diets 27 and 28 with 5% or less shrimp meal in their composition. These data suggest that up to about 50% of the shrimp meal in diet K can be replaced by brewer's yeast with no deleterious effect on the growth of the shrimp.

Since no significant differences were found in growth and survival of animals fed diets K and 30, it is possible to replace about 33% of the rice bran in diet K by an equal percentage of corn meal at the expense of only slightly reduced growth. Corn meal in this proportion is a good binder and can be used instead of the more expensive sodium alginate.

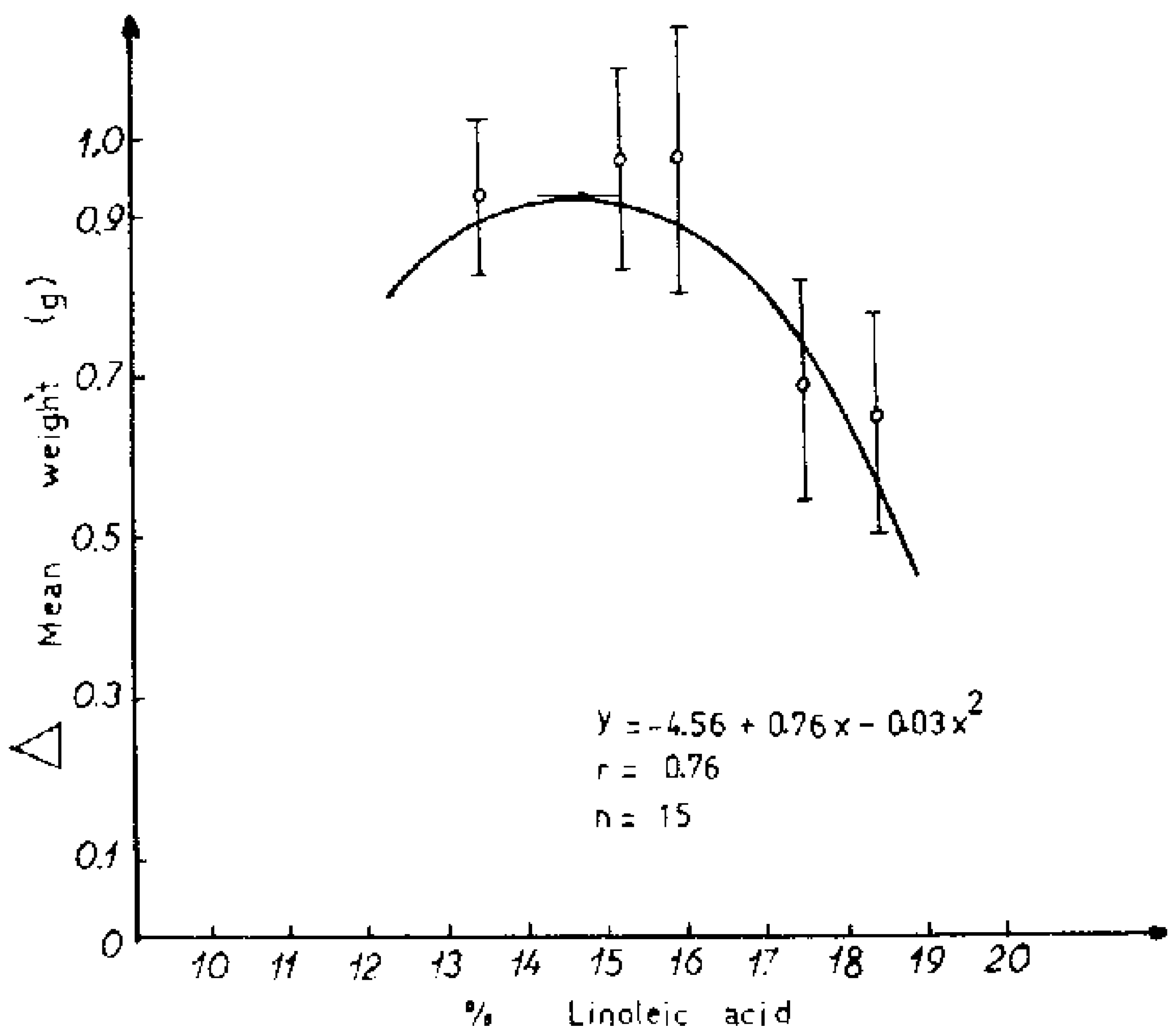

Figure 1. Relation between percentage of linoleic acid in diets fed to *P. stylirostris* and the increase in mean weight. Open circles and bars represent means ± SEM.

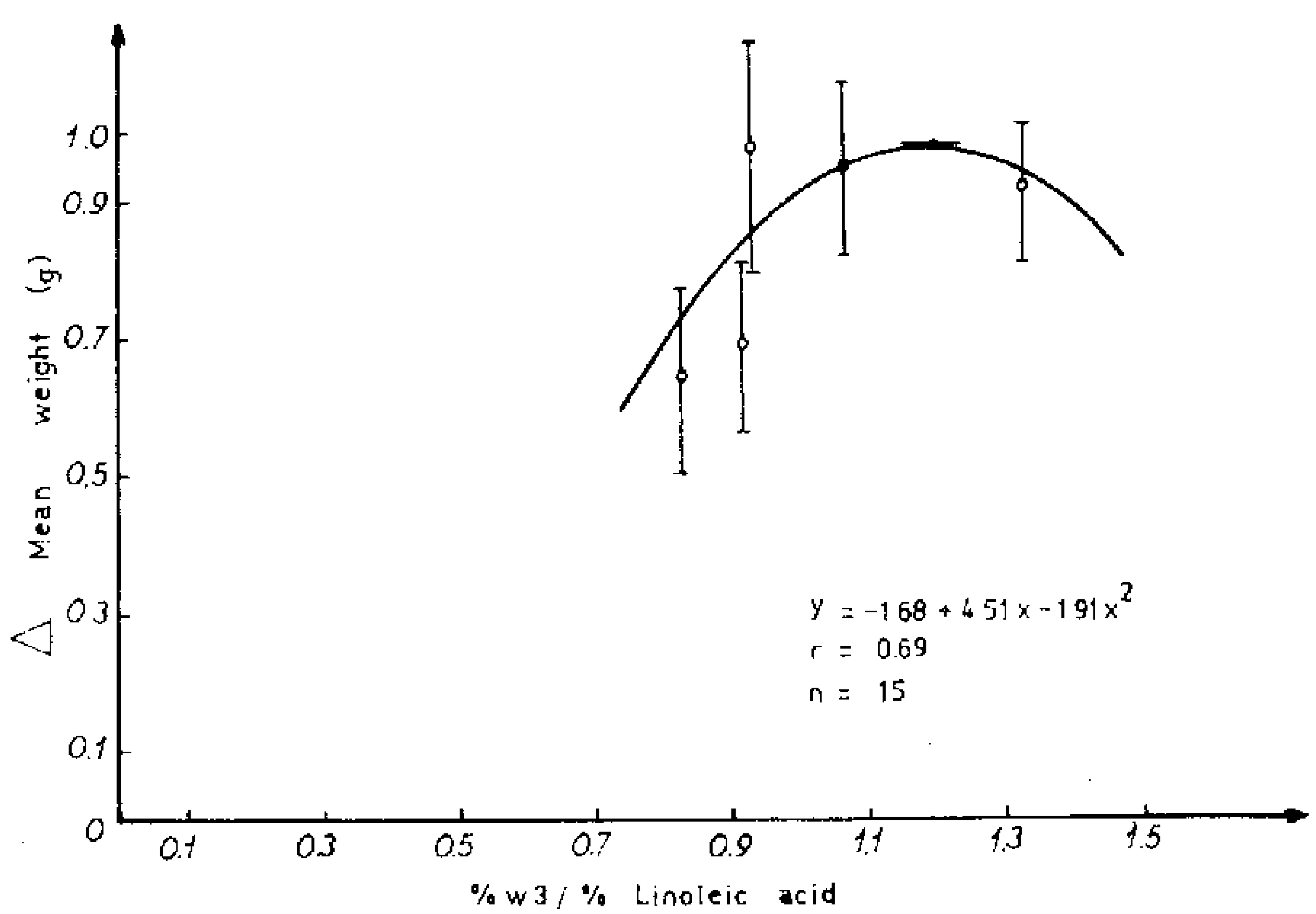

Figure 2. Relation between ratio of percent fatty acid from ω3 series to percent linoleic acid in diets fed to *P. stylirostris* and the increase in mean weight. Open circles and bars represent means ± SEM.

The variations in growth in animals fed diets 25, 26, 27, 28 and K cannot be explained by differences in amount of crude protein and it is unlikely that any multi-ingredient rations for shrimp will be qualitatively deficient in any of the eleven essential amino acids (New 1976). Also, no correlation was observed between growth and total lipid composition of the diets, which varied from 4.1 to 5.6%.

It has been suggested that linoleic and linolenic acids are essential for some fish species, rainbow trout (Castell et al. 1972a,b,c); red sea bream (Yone and Fujii 1975); turbot (Leger et al. 1979) and the silver carp (Csengeri et al. 1978). For penaeid shrimp, not only these two fatty acids but also the 20:5 ω3 and 22:6 ω3 polyunsaturated fatty acids (PUFA) seem to be essential (Shewbart and Mies 1973; Guary et al. 1976; Kanazawa et al. 1977a,b; Colvin 1976; New 1976; Bottino et al. 1980; Clarke and Wickins 1980).

It is interesting to point out that brewer's yeast is reported to have only the following fatty acids: 16:0, 16:1, 18:0, 18:1 and perhaps 18:2 (Shaw 1966), and its content in total lipids is 0.9%. Sun-dried shrimp meal has not only a higher amount of total lipids (5.4%) but also contains some PUFA such as 20:5 and 22:6 acids (Joseph and Meyers 1975). Thus the contribution of these PUFA by yeast is very low for these formulated diets. An increment in percentage of 18:1 ω9 oleic acid was found with the increase of brewer's yeast in the rations (Table 3). Diet K has a relative amount of 17.0% of this acid, while diet 28, in which the 31.5% shrimp meal has been replaced by the same percentage of yeast, showed a 21.8% of oleic acid. Also, the percentage of linoleic acid increased with the level of yeast in the rations: from 13.4% in diet 25 to 18.4% in ration 28. The differences in the relative concentrations of the other fatty acids in the diets are somewhat erratic, and do not follow any logical pattern.

In the experiments reported in this paper, no correlations were found between the gain of weight and the percentage of fatty acids of the ω3 series nor the amount of highly unsaturated fatty acids (20:5 ω3 and 22:6 ω3) probably because they were present in adequate levels and there was only a small variation of these series of compounds in the diets, from 14.8 to 17.9% and 8.9 to 10.2% respectively.

Nevertheless, quadratic relations were found between the increase in mean weight and both the relative percentage of linoleic acid and the ratio % ω3/% linoleic acid in the diets. These results suggest not only the best percentage of linoleic acid for this series of diets but also the importance of the ratio % ω3/% linoleic acid for shrimps, which has been stressed by Sick and Andrews (1973), Colvin (1976) and New (1976). The influence of this ratio on the growth of shrimp could be the result of interactions between the members of the ω3 and ω6 series, as has been suggested for mammals by Holman (1964), for marine flatfish (Owen et al. 1972) and for shrimps (Colvin 1976; New 1976; Deshimaru et al. 1979). Holman also stated that the presence of an excess of fatty acids of the ω6 series inhibited the chain elongation of ω3 acids and vice versa, but in conclusion, the results obtained in this series of experiments indicate that a good diet for juvenile *P. stylirostris* should include at least 15% shrimp meal and less than 15% brewer's yeast, with a level of about 14.5% for 18:2 ω6 and a ratio for % ω3 to % linoleic acid around 1.2.

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